

AMENDMENTS TO THE CLAIMS:

This listing of claims will replace all prior versions, and listings, of claims in the application:

LISTING OF CLAIMS:

Claim 1 (Currently amended): A method for producing a recombinant glycoprotein in a uni- or multicellular fungal host cell which includes an α -1,2-mannosidase activity and a GlcNAc transferase I (GnT I) activity and is diminished or depleted in the activity of an initiating α -1,6-mannosyltransferase ~~and which produces N-glycans comprising GlcNAcMan₅GlcNAc₂ structures~~, the method comprising the step of expressing in the host cell a nucleic acid encoding a chimeric mannosidase enzyme comprising

(a) a *D. melanogaster* mannosidase II catalytic domain fused to a cellular targeting signal peptide selected from the group consisting of Glsl-s, Mns1-s, Mns1-m, S.Sec-s, S.Sec-m, S.Sec-l, P.Sec-s, P.Sec-m, Mnn9-s, Van1-s, Van1-m, Van1-l, Anp1-s, Anp1-m, Anp1-l, Hoc1-s, Hoc1-m, Hoc1-l, Mnn10-m, Mnn11-s, Mnt1-m, J3-m, Ktr1-s, Ktr2-s, Gnt1-s, Gnt1-m, Gnt1-l, Mnn2-s, Mnn2-m, Mnn2-l, Mnn5-m, Mnn1-s, Mnn1-m, Mnn1-l, Mnn6-s, and Mnn6-m or

(b) a *C. elegans* mannosidase II catalytic domain fused to a cellular targeting signal peptide selected from the group consisting of Glsl-s, Mns1-s, Mns1-m, S.Sec-s, S.Sec-m, S.Sec-l, P.Sec-s, Van1-s, Van1-m, Van1-l, Anp1-s, Hoc1-m, Mnn10-s, Mnn10-m, Mnn10-l, Mnn11-s, Mnn11-m, Mnt1-s, Mnt1-m, Mnt1-l, D2-s, D2-m, D9-m, J3-m, Ktr2-s, Gnt1-s, Gnt1-m, Mnn2-s, Mnn2-m, Mnn2-l, Mnn5-s, Mnn5-m, Mnn1-s, Mnn1-m, and Mnn6-m, wherein said chimeric enzyme mannosidase in (a) or (b) is capable of hydrolyzing *in vivo* more than 40-50 percent of the Man α -1,3 and/or Man α -1,6 linkages of a GlcNAcMan₅GlcNAc₂ oligosaccharide substrate,

whereby expression of said chimeric mannosidase produces one or more ~~desired N-glycan~~ N-glycan structures on a recombinant glycoprotein expressed in said host cell wherein the ~~desired N-glycan~~ N-glycan is characterized as having at least the oligosaccharide branch Man α 1,3 (Man α 1,6) Man β 1,4-GlcNAc β 1,4-GlcNAc-Asn.

Claim 2 (Currently amended): A method for producing a recombinant glycoprotein in a uni- or multicellular fungal host cell which includes an α -1,2-mannosidase and a GlcNAc transferase I (GnT I) and is diminished or depleted in the activity of an initiating α -1,6-mannosyltransferase ~~and which produces N-glycans comprising GlcNAcMan₅GlcNAc₂ structures~~, the method comprising the step of expressing in the host cell a nucleic acid encoding a chimeric mannosidase enzyme comprising

(a) a *D. melanogaster* mannosidase II catalytic domain fused to a cellular targeting signal peptide selected from the group consisting of Glsl-s, Mns1-s, Mns1-m, S.Sec-s, S.Sec-m, S.Sec-l, P.Sec-s, P.Sec-m, Mnn9-s, Van1-s, Van1-m, Van1-l, Anp1-s, Anp1-m, Anp1-l, Hoc1-s, Hoc1-m, Hoc1-l, Mnn10-m, Mnn11-s, Mnt1-m, J3-m, Ktr1-s, Ktr2-s, Gnt1-s, Gnt1-m, Gnt1-l, Mnn2-s, Mnn2-m, Mnn2-l, Mnn5-m, Mnn1-s, Mnn1-m, Mnn1-l, Mnn6-s, and Mnn6-m or

(b) a *C. elegans* mannosidase II catalytic domain fused to a cellular targeting signal peptide selected from the group consisting of Glsl-s, Mns1-s, Mns1-m, S.Sec-s, S.Sec-m, S.Sec-l, P.Sec-s, Van1-s, Van1-m, Van1-l, Anp1-s, Hoc1-m, Mnn10-s, Mnn10-m, Mnn10-l, Mnn11-s, Mnn11-m, Mnt1-s, Mnt1-m, Mnt1-l, D2-s, D2-m, D9-m, J3-m, Ktr2-s, Gnt1-s, Gnt1-m, Mnn2-s, Mnn2-m, Mnn2-l, Mnn5-s, Mnn5-m, Mnn1-s, Mnn1-m, and Mnn6-m, wherein said chimeric ~~enzyme~~ mannosidase in (a) and (b) is capable of hydrolyzing *in vivo* more than 40-50 percent of the Man α -1,3 and/or Man α -1,6 linkages of a GlcNAcMan₅GlcNAc₂ oligosaccharide substrate,

whereby expression of said chimeric mannosidase produces one or more ~~desired N-glycan~~ N-glycan structures on a recombinant glycoprotein expressed in said host cell, wherein the ~~desired N-glycan~~ N-glycan is produced within the host cell at a yield of at least 10 mole percent and wherein the ~~desired N-glycan~~ N-glycan is characterized as having at least the oligosaccharide branch Man α 1,3 (Man α 1,6) Man β 1,4-GlcNAc β 1,4-GlcNAc-Asn.

Claims 3-5 (Cancelled)

Claim 6 (Original): The method of claim 1 or 2, wherein the oligosaccharide substrate is characterized as Man α 1,3 (Man α 1,6 Man α 1,6) Man β 1,4-GlcNAc β 1,4-GlcNAc-Asn; Man α 1,3 (Man α 1,3 Man α 1,6) Man β 1,4-GlcNAc β 1,4-GlcNAc-Asn; GlcNAc β 1,2 Man α 1,3 (Man α 1,6 Man α 1,6) Man β 1,4-GlcNAc β 1,4-GlcNAc-Asn; GlcNAc β 1,2 Man α 1,3 (Man α 1,3 Man α 1,6)

Man β 1,4-GlcNAc β 1,4-GlcNAc-Asn; Man α 1,3 (Man α 1,3 Man α 1,6 Man α 1,6) Man β 1,4-GlcNAc β 1,4-GlcNAc-Asn; GlcNAc β 1,2 Man α 1,3 (Man α 1,3 Man α 1,6 Man α 1,6) Man β 1,4-GlcNAc β 1,4-GlcNAc-Asn; Man α 1,2 Man α 1,3 (Man α 1,3 Man α 1,6 Man α 1,6) Man β 1,4-GlcNAc β 1,4-GlcNAc-Asn; Man α 1,2 Man α 1,3 (Man α 1,3 Man α 1,6) Man β 1,4-GlcNAc β 1,4-GlcNAc-Asn; Man α 1,2 Man α 1,3 (Man α 1,6 Man α 1,6) Man β 1,4-GlcNAc β 1,4-GlcNAc-Asn or high mannan.

Claim 7-9 (Cancelled)

Claim 10 (Currently amended): The method of claim 1 or 2, wherein the chimeric mannosidase comprises a Class IIx mannosidase catalytic domain fused to a cellular targeting signal peptide that targets the chimeric ~~enzyme~~ mannosidase to the secretory pathway of the host cell.

Claim 11 (Previously presented) : The method of claim 10, wherein the Class IIx mannosidase enzyme has a substrate specificity for Man α 1,3 (Man α 1,6 Man α 1,6) Man β 1,4-GlcNAc β 1,4-GlcNAc-Asn; Man α 1,3 (Man α 1,3 Man α 1,6) Man β 1,4-GlcNAc β 1,4-GlcNAc-Asn; or Man α 1,2 Man α 1,3 (Man α 1,3 Man α 1,6 Man α 1,6) Man β 1,4-GlcNAc β 1,4-GlcNAc-Asn.

Claim 12 (Currently amended): The method of claim 1 or 2, wherein the chimeric mannosidase enzyme comprises a Class III mannosidase catalytic domain fused to a cellular targeting signal peptide that targets the chimeric ~~enzyme~~ mannosidase to the secretory pathway of the host cell.

Claim 13 (Currently amended) : The method of claim 12, wherein the Class III mannosidase enzyme has a substrate specificity for Man α 1,3 (Man α 1,6 Man α 1,6) Man β 1,4-GlcNAc β 1,4-GlcNAc-Asn; Man α 1,3 (Man α 1,3 Man α 1,6) Man β 1,4-GlcNAc β 1,4-GlcNAc-Asn; Man α 1,3 (Man α 1,3 Man α 1,6 Man α 1,6) Man β 1,4-GlcNAc β 1,4-GlcNAc-Asn; or high mannans.

Claim 14 (Currently amended) : The method of claim 1 or 2, wherein the chimeric mannosidase enzyme is overexpressed.

Claim 15 (Currently amended) : The method of claim 1 or 2, wherein the chimeric mannosidase enzyme is further capable of hydrolyzing a Man α 1,2 linkage.

Claim 16 (Currently amended) : The method of claim 1 or 2, wherein the chimeric mannosidase enzyme has a pH optimum of from about 5.0 to about 8.0.

Claim 17 (Canceled)

Claim 18 (Currently amended) : The method of claim 1 or 2, wherein the chimeric mannosidase enzyme is localized within the secretory pathway of the host cell.

Claim 19 (Currently amended) : The method of claim 1 or 2, wherein the chimeric mannosidase enzyme is localized within at least one of the ER, Golgi apparatus or the trans Golgi network of the host cell.

Claims 20-25 (Cancelled)

Claim 26 (Original): The method of claim 1 or 2, further comprising the step of isolating the glycoprotein from the host cell.

Claim 27 (Original) : The method of claim 1 or 2, wherein the host cell is selected from the group consisting of *Pichia pastoris*, *Pichia finlandica*, *Pichia trehalophila*, *Pichia koclamae*, *Pichia membranaefaciens*, *Pichia opuntiae*, *Pichia thermotolerans*, *Pichia salictaria*, *Pichia guercuum*, *Pichia pijperi*, *Pichia stiptis*, *Pichia methanolica*, *Pichia sp.*, *Saccharomyces cerevisiae*, *Saccharomyces sp.*, *Hansenula polymorpha*, *Kluyveromyces sp.*, *Kluyveromyces lactis*, *Candida albicans*, *Aspergillus nidulans*, *Aspergillus niger*, *Aspergillus oryzae*, *Trichoderma reesei*, *Chrysosporium lucknowense*, *Fusarium sp.*, *Fusarium gramineum*, *Fusarium venenatum* and *Neurospora crassa*.

Claim 28 (Original) : The method of claim 27, wherein the host cell is *Pichia pastoris*.

Claim 29 (Original) : The method of claim 1 or 2, wherein the glycoprotein is a therapeutic protein.

Claim 30 (Original) : The method of claim 29, wherein the therapeutic protein is selected from the group consisting of erythropoietin, cytokines, coagulation factors, soluble IgE receptor α -chain, IgG, IgG fragments, IgM, interleukins, urokinase, chymase, urea trypsin inhibitor, IGF-

binding protein, epidermal growth factor, growth hormone-releasing factor, annexin V fusion protein, angiostatin, vascular endothelial growth factor-2, myeloid progenitor inhibitory factor-1, osteoprotegerin, α -1-antitrypsin and α - feto protein.

Claims 31 – 56 (Cancelled)

Claim 57 (Currently amended) : The method of claim 1, wherein the ~~desired N-glycan~~ N-glycan comprises an oligosaccharide structure selected from the group consisting of Man₃GlcNAc₂, GlcNAcMan₃GlcNAc₂, and Man₄GlcNAc₂.

Claim 58 (Currently amended): The method of claim 2, wherein the ~~desired N-glycan~~ N-glycan comprises an oligosaccharide structure selected from the group consisting of Man₃GlcNAc₂, GlcNAcMan₃GlcNAc₂, and Man₄GlcNAc₂.

Claim 59 (New): A method for producing a recombinant glycoprotein in a yeast host cell comprising:

(a) expressing a nucleic acid encoding the recombinant glycoprotein in a yeast host cell that is diminished or depleted in the activity of an initiating α -1,6-mannosyltransferase and expresses an α -1,2-mannosidase activity, a GlcNAc transferase I (GnT I) activity, and a chimeric mannosidase enzyme comprising

(i) a *D. melanogaster* mannosidase II catalytic domain fused to a cellular targeting signal peptide selected from the group consisting of Glsl-s, Mns1-s, Mns1-m, S.Sec-s, S.Sec-m, S.Sec-l, P.Sec-s, P.Sec-m, Mnn9-s, Van1-s, Van1-m, Van1-l, Anp1-s, Anp1-m, Anp1-l, Hoc1-s, Hoc1-m, Hoc1-l, Mnn10-m, Mnn11-s, Mnt1-m, J3-m, Ktr1-s, Ktr2-s, Gnt1-s, Gnt1-m, Gnt1-l, Mnn2-s, Mnn2-m, Mnn2-l, Mnn5-m, Mnn1-s, Mnn1-m, Mnn1-l, Mnn6-s, and Mnn6-m or

(ii) a *C. elegans* mannosidase II catalytic domain fused to a cellular targeting signal peptide selected from the group consisting of Glsl-s, Mns1-s, Mns1-m, S.Sec-s, S.Sec-m, S.Sec-l, P.Sec-s, Van1-s, Van1-m, Van1-l, Anp1-s, Hoc1-m, Mnn10-s, Mnn10-m, Mnn10-l, Mnn11-s, Mnn11-m, Mnt1-s, Mnt1-m, Mnt1-l, D2-s, D2-m, D9-m, J3-m, Ktr2-s, Gnt1-s, Gnt1-m, Mnn2-s, Mnn2-m, Mnn2-l, Mnn5-s, Mnn5-m, Mnn1-s, Mnn1-m, and Mnn6-m

wherein said chimeric mannosidase in (a) or (b) is capable of hydrolyzing *in vivo* more than 40-50 percent of the Man α -1,3 and/or Man α -1,6 linkages of a GlcNAcMan₅GlcNAc₂ oligosaccharide substrate and wherein the recombinant glycoprotein expressed in said host cell comprises *N*-glycans characterized as having at least the oligosaccharide branch Man α 1,3 (Man α 1,6) Man β 1,4-GlcNAc β 1,4-GlcNAc-Asn; and

(b) isolating the recombinant glycoprotein.

Claim 60 (New): The method of claim 59, wherein the chimeric mannosidase comprises a Class IIx mannosidase catalytic domain fused to a cellular targeting signal peptide that targets the chimeric mannosidase to the secretory pathway of the host cell.

Claim 61 (New): The method of claim 10, wherein the Class IIx mannosidase enzyme has a substrate specificity for Man α 1,3 (Man α 1,6 Man α 1,6) Man β 1,4-GlcNAc β 1,4-GlcNAc-Asn; Man α 1,3 (Man α 1,3 Man α 1,6) Man β 1,4-GlcNAc β 1,4-GlcNAc-Asn; or Man α 1,2 Man α 1,3 (Man α 1,3 Man α 1,6 Man α 1,6) Man β 1,4-GlcNAc β 1,4-GlcNAc-Asn.

Claim 62 (New): The method of claim 1 or 2, wherein the chimeric mannosidase enzyme comprises a Class III mannosidase catalytic domain fused to a cellular targeting signal peptide that targets the chimeric mannosidase to the secretory pathway of the host cell.

Claim 63 (New) : The method of claim 12, wherein the Class III mannosidase enzyme has a substrate specificity for Man α 1,3 (Man α 1,6 Man α 1,6) Man β 1,4-GlcNAc β 1,4-GlcNAc-Asn; Man α 1,3 (Man α 1,3 Man α 1,6) Man β 1,4-GlcNAc β 1,4-GlcNAc-Asn; Man α 1,3 (Man α 1,3 Man α 1,6 Man α 1,6) Man β 1,4-GlcNAc β 1,4-GlcNAc-Asn; or high mannans..

Claim 64 (New) : The method of claim 1 or 2, wherein the yeast host cell is selected from the group consisting of *Pichia pastoris*, *Pichia finlandica*, *Pichia trehalophila*, *Pichia koclamae*, *Pichia membranaefaciens*, *Pichia opuntiae*, *Pichia thermotolerans*, *Pichia salictaria*, *Pichia guercuum*, *Pichia pijperi*, *Pichia stiptis*, *Pichia methanolica*, *Pichia sp.*, *Saccharomyces cerevisiae*, *Saccharomyces sp.*, *Hansenula polymorpha*, *Kluyveromyces sp.*, *Kluyveromyces lactis*, and *Candida albicans*..

Claim 65 (New) : The method of claim 64, wherein the yeast host cell is *Pichia pastoris*.

Claim 66 (New) : The method of claim 59, wherein the recombinant glycoprotein is a therapeutic protein.

Claim 67 (New) : The method of claim 66, wherein the therapeutic protein is selected from the group consisting of erythropoietin, cytokines, coagulation factors, soluble IgE receptor α -chain, IgG, IgG fragments, IgM, interleukins, urokinase, chymase, urea trypsin inhibitor, IGF-binding protein, epidermal growth factor, growth hormone-releasing factor, annexin V fusion protein, angiostatin, vascular endothelial growth factor-2, myeloid progenitor inhibitory factor-1, osteoprotegerin, α -1-antitrypsin and α - feto protein.